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Quantitative trait loci for aluminum resistance in wheat

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Abstract Quantitative trait loci (QTL) for wheat resistance to aluminum (Al) toxicity were analyzed using simple sequence repeats (SSRs) in a population of 192 F_6 recombinant inbred lines (RILs) derived from a cross between an Al-resistant cultivar, Atlas 66 and an Al-sensitive cultivar, Chisholm. Wheat reaction to Al was measured by relative root growth and root response to hematoxylin stain in nutrient-solution culture. After screening 1,028 SSR markers for polymorphisms between the parents and bulks, we identified two QTLs for Al resistance in Atlas 66. One major QTL was mapped on chromosome 4D that co-segregated with the Al-activated malate transporter gene (*ALMT1*). Another minor QTL was located on chromosome 3BL. Together, these two QTLs accounted for about 57% of the phenotypic variation in hematoxylin staining score and 50% of the variation in net root growth (NRG). Expression of the minor QTL on 3BL was suppressed by the major QTL on 4DL. The two QTLs for Al resistance in Atlas 66 were also verified in an additional RIL population derived from Atlas 66/Century. Several SSR markers closely linked to the QTLs were identified and have potential to be used for marker-assisted selection (MAS) to improve Al-resistance of wheat cultivars in breeding programs.

Keywords *Triticum aestivum* · Aluminum resistance · SSR marker · QTL mapping

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Introduction

Aluminum (Al) is the most abundant metal element in soil, and is released as Al^{3+} to soil solution at pH < 5. Exchangeable Al^{3+} enters the root tip cells to cripple root development of wheat plants. Al toxicity in acidic soils is a major constraint for crop production on about 30–40% of world arable lands (von Uexküll and Mutert 1995). The addition of lime to acidic soils can significantly relieve Al toxicity by increasing soil pH, but the energy costs for application or actual cost of lime often prohibits widespread adoption of this practice. Fortunately, genetic variation in Al resistance exists in wheat (Garvin and Carver 2003), and the adoption of Al resistant cultivars is

often considered the most cost-effective measure to improve wheat production in acidic soils.

The number of genetic loci that control Al resistance in wheat still remains unresolved. Riede and Anderson (1996) reported a single gene on 4DL (Alt_{BH}) segregating for Al resistance in the population, BH 1146× Anahuac. In Chinese Spring, another gene for Al resistance (*Alt2*) was discovered on the same chromosome arm by studying disomic substitution lines (Luo and Dvorak 1996). Several other reports suggested oligogenic control of Al resistance in BH1146 (Delhaize et al. 1993a; Somers and Gustafson 1995; Basu et al. 1997; Milla and Gustafson 2001).

The release of malate from root tips was proposed as an exclusion mechanism for Al resistance in wheat (Delhaize et al. 1993b; Basu et al. 1994; Pellet et al. 1996). More recently, an Al-activated malate transporter gene (*ALMT1*) was cloned (Sasaki et al. 2004) and showed co-segregation with Al resistance in F_2 and F_3 populations derived from a cross between two near-isogenic wheat lines (NILs), ET8 (Al-resistant), and ES8 (Al-sensitive). These NILs were derived from a cross between the Al-resistant cultivar, Carazinho, and the Al-sensitive cultivar, Egret (Fisher and Scott 1987). Incorporation of *ALMT1* into barley by transformation increased Al resistance of barley (Delhaize et al. 2004). These results lend support to major-gene control of Al resistance in wheat.

Other studies indicated that Al resistance in Atlas 66 is determined by a complex genetic mechanism involving more than one gene (Berzonsky 1992). Al-resistant NILs that carry only partial Al resistance from Atlas 66 provides indirect evidence to support this assumption (Carver et al. 1993). In addition, Tang et al. (2002) demonstrated that at least two genetic loci might contribute to Al resistance in Atlas 66.

Although hematoxylin staining of root apices shows a semi-quantitative character for Al resistance, it has been proven to be an easy, rapid, reliable, and non-destructive method for discerning among Al-resistant and Al-sensitive genotypes. Hematoxylin turns dark purple when it forms a complex with Al so that the penetration and retention of Al ion in the roots can be assessed and the reaction between hematoxylin and Al is specific (Cançado et al. 1999). To get a comprehensive picture of Al resistance, in the study presented here, hematoxylin staining of roots was used in addition to root elongation measurements to identify resistant and sensitive wheat genotypes. Our objectives were to validate a major Quantitative trait loci (QTL) for Al resistance on chromosome 4D in a different mapping population, identify new QTL for Al resistance from Atlas 66, and develop high-throughput PCR-based markers for marker-assisted selection (MAS) of Al-resistance QTLs in breeding programs.

Materials and methods

Plant materials and evaluation of Al resistance

The mapping population used in this study contained 192 F6 recombinant inbred lines (RILs) derived by single-seed-descent from a cross between Atlas 66 and the Al-sensitive cultivar, Chisholm. In addition, six NILs derived from Atlas 66/3*Century (OK91G103, OK91G104, OK91G107) and from Atlas 66/3*Chisholm (OK91G105, OK91G106, OK91G108) were evaluated for markers linked to Al resistance that were mapped in the RILs.

To evaluate Al resistance of the RILs, wheat seeds were placed on moistened filter paper in a petri dish at 4°C for 24 h, and then moved to room temperature (22–25°C) for an additional 24 h. Three germinated seeds with similar viability were transferred onto a nylon net at the open bottom of a plastic cup. The cups were supported by a plastic cup holder floating in de-ionized water at 22°C with 16 h fluorescent light daily. Two bubble rods in the water connected to an air pump provided aeration during the culture period. After 48 h hydroponic culture, the deionized water was replaced with a nutrient solution (pH 4.0) consisting of 4 mM $CaCl_2$, 6.5 mM KNO_3, 2.5 mM $MgCl_2.6H_2O$, 0.4 mM NH_4NO_3, 0.1 mM $(NH4)_2SO_4$, and 0.36 mM $AlK(SO_4).2H_2O$. The Al-free treatment did not include the addition of $AlK(SO_4).2H_2O$.

Al reactions of parents and RILs were evaluated by measuring root growth during Al stress and the degree of hematoxylin staining of Al-treated root tips. The principal root of each seedling was measured after growing in the deionized water for the first 48 h. After 48 h Al exposure, the same root was measured again. The difference between the two measurements was calculated as net root growth (NRG) for Al-treated seedlings and control root growth (CRG) for the non-Al-treated seedlings. Following the root length measurements, excess Al^{3+} on the root surface was rinsed off in deionized water for 1 h, with 2–3 water replacements. Clean roots were then submerged in a hematoxylin solution containing 0.2% hematoxylin (w/v) and 0.02% (w/v) KIO_3 for 15 min, followed by rinsing the roots with deionized water 3–4 times. Root tips of each stained seedling were visually scored as three grades: no stain on root tips as grade 1, light stain as grade 2, and heavy stain as grade 3. The experiment was repeated twice at different times in a growth room with controlled temperature and light length and analyzed as a randomized complete block design.

Marker analysis

After hematoxylin staining, wheat seedlings from the first experiment were grown in the nutrient solution for about one more week to generate leaf tissue for DNA isolation. Leaf tissue from each line was collected in a 1.5-mL tube and dried in a freeze-drier for 2 days. The tubes with dried tissue were shaken at 30 times/s for 3 min using a Mixer Mill (Retsch GmbH, Haan, Germany) with a 3.2 mm stainless bead in each tube. DNA was extracted using the cetyltrimetryl ammonium bromide (CTAB) method (Saghai-Maroof et al. 1984).

Bulked segregant analysis was used to screen polymorphic simple sequence repeat (SSR) markers associated with Al resistance. One bulk consisted of six highly Al-resistant RILs and the other was derived from six highly Al-sensitive RILs. The two bulks and parents were screened with 1,028 pairs of SSR primers for polymorphism, which included 500 BARC primers (Song et al. 2005), 361 WMC primers (Somers et al. 2004), 103 CFD and 36 CFA primers (Guyomarc'h et al. 2002; Sourdille et al. 2003), 22 GWM primers (Roder et al. 1998), and six GDM primers (Pestsova et al. 2000). Polymorphic markers between the parents and between the bulks were further analyzed in the F_6 RIL population.

The PCR were performed as described by Ma et al. (2005) in a DNA Engine Tetrad® Peltier Thermal Cycler (Bio-Rad Lab, Hercules, CA, USA). The primers and PCR protocol from Sasaki et al. (2004) were used to amplify the *ALMT1* gene marker. PCR products (2 μL) were subjected to digestion overnight with 5 units of *Xmn* I in a 20 μL of reaction volume. The digested products were separated on a 2% agarose gel with 1× TAE buffer under 80 V voltages for 20 min and visualized under UV light.

Data analysis

Broad-sense heritability (h^2) was computed as $\sigma_g^2/\sigma_g^2 + \sigma_g^2$ using SAS® Version 9.1 (SAS Institute Inc., 2003, Cary, NC, USA), where σ_g^2 and σ_e^2 were the estimates of genetic and error variances, respectively. Other statistical analyses were performed by GLM procedure with LSMEANS option. Linkage analysis of SSR markers was conducted using JoinMap®3.0 (Van Ooijen and Voorrips 2001) with an LOD score of 3.0. MapQTL® 5 (Van Ooijen 2004) was used for interval mapping of QTL and estimation of determination coefficient (R^2).

Results

Phenotypic variation in root growth rate during Al stress

In the growth medium with 0.36 mM Al^{3+}, roots of Atlas 66 were longer (3.2 cm) than those of Chisholm (0.4 cm) after 2 days of nutrient-solution culture (Table 1). Therefore, the Al concentration used in this study was considered adequate for differentiating resistant versus susceptible lines. Compared to the non-Al control treatment, Al stress caused a 24% decrease in root elongation for Atlas 66 but a 92.4% decrease for Chisholm. The frequency distribution of NRG of the Al-treated RILs during Al-stress formed

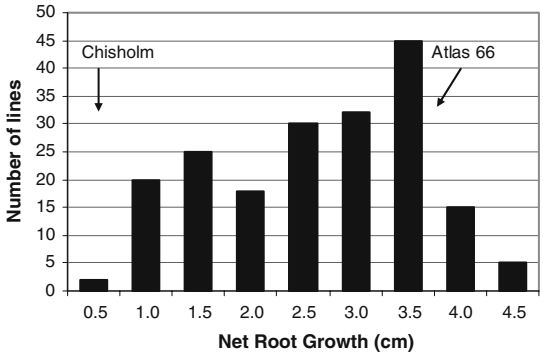

Fig. 1 Frequency distribution of net root growth (*NRG*) during Al treatment from RILs and two parents, Atlas66 and Chisholm

two peaks with the larger one toward Atlas 66 (Fig. 1). The broad-sense heritability for NRG during Al treatment was relatively high (Table 1).

The hematoxylin stain produced no stain on the root tips of Atlas 66 (grade 1) but complete stain on Chisholm (grade 3). The average hematoxylin stain score (HSS) for the RIL population was 1.7. When the RILs were grouped based on their HSS, the average NRG decreased 1 cm as HSS increased 1 grade. The differences in NRG among the three HSS groups of RILs were highly significant ($p < 0.01$, Fig. 2).

QTL mapping

After 1,028 SSR markers were screened, 253 primers amplified at least one polymophic band between Atlas 66 and Chisholm, and 50 of them were polymorphic between the two bulks. Further analysis of the 50 primers in the population of RILs identified two linkage groups: one with five SSR markers spanning 57.9 cM on chromosome 4DL, and another spanning 16.7 cM with seven SSRs on 3BL (Fig. 3), based on previous mapped SSR markers.

Interval mapping identified two QTLs for Al resistance on the two chromosomes (Fig. 3). The QTL on 4DL that co-segregated with *ALMT1* showed a major effect on both NRG and HSS, whereas QTL on 3BL had a smaller effect on both measurements of Al resistance (Table 2). SSR marker Xbarc164 was the closest marker to the QTL on 3BL, with a LOD value exceeding 3. A flanking marker was not found for this QTL (Fig. 3a). The QTL on 3BL was validated in another RIL population of Atlas 66/Century with a R^2 of 12.8 and LOD value of 3.34 based on HSS. The association between Al resistance and the remaining markers identified in bulk segregant analysis was not significant.

To analyze the effect of the two QTLs on Al resistance, markers with the highest R^2, i.e., *ALMT1* gene marker on 4DL and the SSR Xbarc164 on 3BL, were selected to represent the two QTLs. Four possible allelic combinations of these two QTLs are: $4DL^+/3BL^+$, $4DL^+/3BL^-$, $4DL^-/3BL^+$, $4DL^-/3BL^-$, in which $4DL^+$ and $3BL^+$ represent Atlas 66 alleles of QTLs on 4DL or 3BL, respectively, and $4DL^-$ and $3BL^-$ represent Chisholm alleles of QTLs on 4DL and 3BL, respectively. Mean comparisons of these genotypic classes indicated that the resistant allele on 4DL alone increases NRG by about 2 cm relative to the genotype with neither resistant allele on 4DL or 3BL (Fig. 4a). The Atlas 66 allele on 3BL did not provide any additional Al resistance in the

Table 1 Parameters of Al resistance for the RIL population and their parents

	CRG (cm)	NRG (cm)	HSS
Atlas 66	4.27 ± 0.61	3.24 ± 0.65	1
Chisholm	5.13 ± 0.63	0.39 ± 0.19	3
Mean of RILs	4.63 ± 0.47	2.39 ± 0.63	1.7
Range of RILs	1.83–7.20	0.36–4.63	1–3
H^2 for RILs (%)	72.44	68.17	80.85
$LSD_{0.01}$	2.27	1.15	0.6
CV for RILs (%)	10.21	26.39	20.42

NRG net root growth, *HSS* hematoxylin staining score, h^2 broad sense heritability, mean ± SE

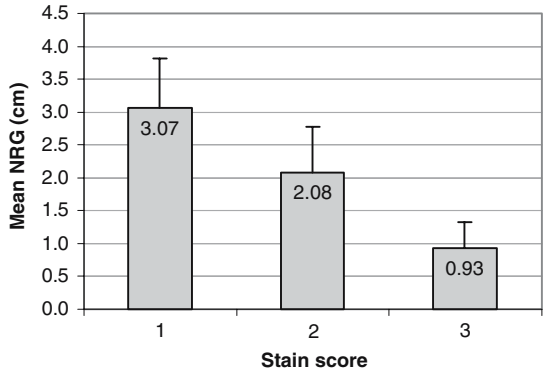

Fig. 2 Mean NRG based on hematoxylin staining scores from RILs of Atlas66/Chisholm. Means were computed as Least Squares Means (*LSMEAN*) in multiple comparison. Least Significant Difference (*LSD*) at $p = 0.01$ is 0.17

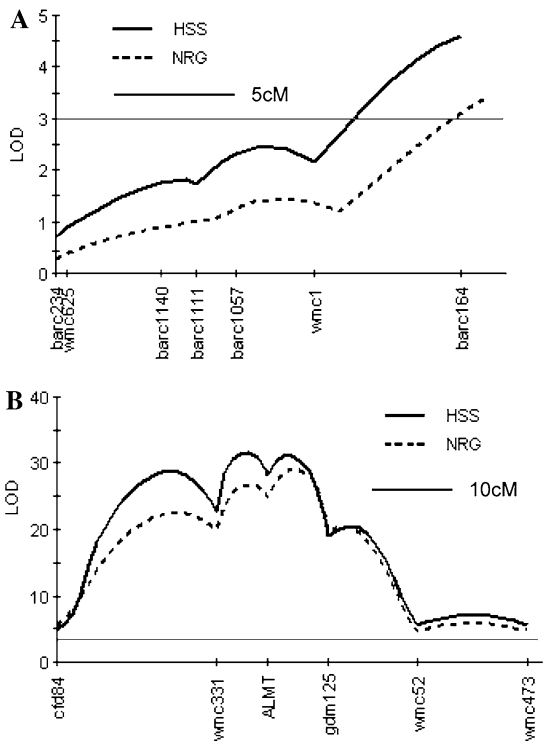

Fig. 3 Interval analysis of QTLs associated with Aluminum resistance based on NRG and HSS. **a** Major QTL on 4DL, **b** Minor QTL on 3BL

presence of the Atlas 66 allele on 4DL. However, in the absence of the Atlas 66 allele on 4DL, the resistant allele on 3BL increased NRG by 1 cm,

Table 2 Effect of QTLs as measured by net root growth (*NRG*) and hematoxylin staining score (*HSS*) on 4DL and 3BL derived from RIL population of Atlas 66/Chisholm

Markers	NRG		HSS	
	LOD	R^2	LOD	R^2
Xbarc164	3.58	8.6	4.61	11.1
Xwmc331	19.94	38	22.59	41.9
ALMT1	24.85	43.1	28.15	49.1
Xgdm125	19.4	37.3	19.12	37.1
Combined		49.6		57.6

and the average hematoxylin score is 1.87, which is an intermediate grade for hematoxylin staining.

Six NILs were also evaluated with SSR markers Xwmc331 and Xgdm125 for the 4DL QTL and Xbarc164 for the 3BL QTL (Table 3). Previous studies demonstrated that the NILs OK91G103, OK91G104, OK91G105, OK91G106 were Al resistant but slightly less resistant than Atlas 66. The NILs OK91G107 and OK91G108 were Al sensitive (Carver et al. 1993; Tang et al. 2002). Most NILs had the same marker allele on 3BL as Chisholm (or Century), except OK91G105 had the Atlas 66 marker allele. For the major QTL on 4DL, Atlas 66 alleles for Xwmc331 and Xgdm125 appeared in all Al-resistant NILs, and the Chisholm (or Century) alleles appeared in two Al-sensitive NILs. Hence, the NILs were essentially differentiated on the basis of the 4DL QTL, not the 3BL QTL.

Effect of *ALMT1* gene on Al-resistance

The *ALMT1* gene has two alleles, *ALMT1*-1 (resistant) and *ALMT1*-2 (sensitive). The primers for *ALMT1* amplify a 107-bp DNA fragment (*ALMT1*-1). The *ALMT1*-2 allele has an *Xmn* I restriction site, which partitions the PCR product into two small fragments of 57- and 50-bp, respectively. Linkage analysis indicated that the *ALMT1* marker co-segregated with the major QTL on 4DL and was located between SSR markers Xgdm125 and Xwmc331. Genetic distances between *ALMT1* and the two flanking markers were 6.3 cM (Xwmc331) and 7.4 cM (Xgdm125), respectively. The R^2 for *ALMT1* was 43.1% for NRG and 49.1% for HSS.

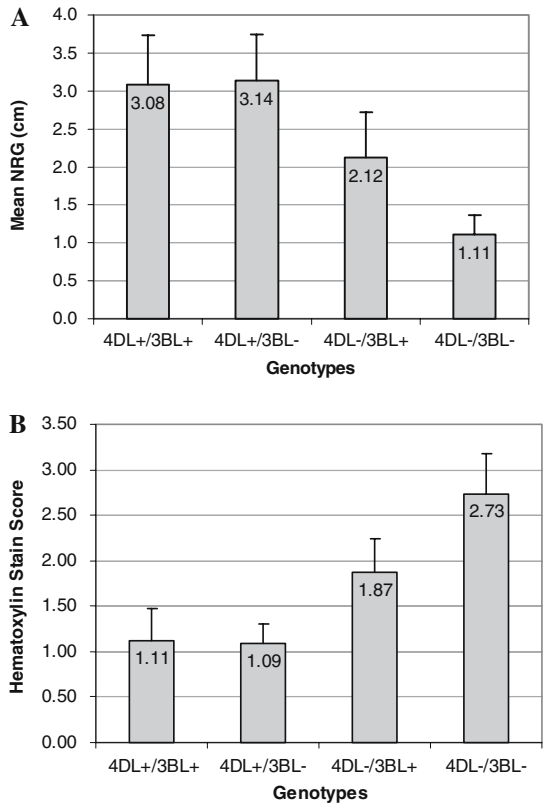

Fig. 4 Effects of the combination of two QTLs based on RILs from Atlas66/Chisholm. "*4DL+/3BL+*" indicates a plant carries both Al-resistance alleles on 4DL and 3BL; "*4DL+/3BL-*" indicates a plant carries Al-resistance alleles on 4DL only; "*4DL-/3BL+*" indicates a plant carries Al-resistance alleles on 3BL only; "*4DL-/3BL-*" indicates a plant carries none of Al-resistance alleles on 4DL and 3BL. Means were computed as Least Squares Means (*LSMEANS*) in multiple comparison. **a** Effects based on NRG. The difference between $mean_{(4DL+/3BL+)}$ and $mean_{(4DL+/3BL-)}$ is not significant, whereas the differences among $mean_{(4DL+/3BL\pm)}$, $mean_{(4DL-/3BL+)}$ and $mean_{(4DL-/3BL-)}$ are significant at $p = 0.01$ ($LSD_{0.01} = 0.34$). **b** Effects based on HSS. The difference between $mean_{(4DL+/3BL+)}$ and $mean_{(4DL+/3BL-)}$ is not significant, the differences among $mean_{(4DL+/3BL\pm)}$, $mean_{(4DL-/3BL+)}$, and $mean_{(4DL-/3BL-)}$ are significant at $p = 0.01$ ($LSD_{0.01} = 0.24$)

Discussion

The ratio of NRG of Al-stressed seedlings to NRG of non-Al-stressed controls has been used to measure plant resistance to Al toxicity in several studies (Taylor and Foy 1985; Parker and Pedler 1998), but this measurement could underestimate Al-resistance for those plants sensitive to low pH per se (without Al) or overestimate the resistance of plants that have more resistant to low pH (Vitorello et al. 2005). To avoid this potential bias, two measurements of Al resistance were used in this study: NRG during Al stress and HSS of Al-treated roots.

Hematoxylin, a natural dye, cannot directly stain tissue successfully because it is necessary to include a mordant, e.g., Al or iron, to form a dye-mordant (D-M) complex for efficiency. With mild alkaline treatment these complexes are converted into a neutral chelate, which gives a purple color and are attracted to negatively charged sites, displaying a particular affinity for polyphosphates. In the case of Al-hematoxylin complexes, the major tissue-binding site is thought to be the phosphoric acid residue in nucleic acid (nuclear DNA/cytoplasmic and nucleic RNA) with the linkage, at least initially, being electrostatic and occurring through the Al ion (Scott and Willett 1966; Bettinger and Zimmermann 1991). One study showed that Al can indeed accumulate in the nucleus (Silva et al. 2000), even at low-Al concentrations for a short-exposure period. This hematoxylin staining technique measures the extent of Al accumulation in root cells and has been widely used to evaluate Al resistance in several crops (Delhaize et al. 1993a; Cançado 1999; Anas 2000). We were able to map QTL for HSS and NRG to the same chromosome position, with a slight higher LOD score for HSS. The two QTLs on 4DL and 3BL together accounted for about 58% of the phenotypic variation for HSS and 50% for NRG. While both HSS and NRG are informative of Al resistance in wheat, HSS may be more accurate for measuring Al resistance of wheat in nutrition solution. This observation is consistent with previous results (Ma et al. 2005). In addition, HSS is simpler, less prone to environmental variation, and less labor-intensive than direct root length measurement, and therefore it is more practical for large-scale screening of Al resistance of wheat in laboratory conditions.

The inheritance of Al resistance in wheat has been extensively studied. Kerridge and Kronstad (1968) reported that a single dominant gene was responsible for Al-resistance in the cross Druchamp/Brevor. Riede and Anderson (1996) mapped one gene (Alt_{BH}) for Al resistance on 4DL of BH 1146 using RFLP and concluded that

Table 3 Segregation patterns for markers Xwmc331, Xgdm125, and Xbarc164 in NILs

Lines	Pedigree	Reaction to Al toxicity	Size of target band (bp)		
			Xwmc331	Xgdm125	Xbarc164
Atlas66		Resistant	151	159	203
Chisholm		Susceptible	149	161	222
OK91G103	Chisholm *4/Atlas 66	Partial resistant	151	159	222
OK91G104	Chisholm *4/Atlas 66	Partial resistant	151	159	222
OK91G105	Century *4/Atlas 66	Partial resistant	151	159	203
OK91G106	Century *4/Atlas 66	Partial resistant	151	159	222
OK91G107	Chisholm *4/Atlas 66	Susceptible	149	161	222/203
OK91G108	Century *4/Atlas 66	Susceptible	149	161	222

this gene was fully responsible for Al resistance in BH 1146. Another gene, *Alt2* from Chinese Spring, was mapped at a similar location as Alt_{BH} (Luo and Dvorak 1996). Our previous work identified a QTL for Al resistance on 4DL of Atlas 66 in the RIL population from the cross, Atlas 66/Century (Ma et al. 2005). This QTL showed a major effect for Al resistance and explained about 50% of the phenotypic variation. The same result was obtained in this study with the RIL population from the cross, Atlas 66/Chisholm, indicating that the QTL on 4DL is a major QTL for Al resistance and highly expressed in different genetic backgrounds.

More recently, Sasaki et al. (2004) cloned a wheat *ALMT1*, which directly supports the hypothesis that wheat resistance to Al toxicity is mainly conditioned by rapid release of malate from root tips as an Al^{3+}-chelating agent. This gene has been mapped on 4DL in our previous study and coincides with a major QTL on 4DL for Al resistance in the RIL population, Atlas 66/Century (Ma et al. 2005). The current study further validated this result in a different RIL population (Atlas 66/Chisholm). The *ALMT1* gene marker showed the highest R^2-value for both NRG and HSS among several linked markers on 4DL, indicating *ALMT1* is an important gene for Al resistance on 4DL of Atlas 66.

The QTL for Al resistance on 4DL has been reported in different genotypes such as BH 1146 and ET8. However, examination of pedigrees reveals a putatively common source of resistance in Polyssu, a Brazilian cultivar that has a high level of Al resistance. BH 1146 (Ponta Grossa 1// Fronteira/Mentana) and Atlas 66 (Frondoso// Redhart 3/Noll 28) both contain Polyssu in their pedigree, as Ponta Grossa 1 was a selection from Polyssu and Frondoso was derived from the cross, Polyssu/Alfredo Chaves 6. The Al-resistant NIL ET8 used for cloning of the *ALMT1* gene was derived from a Brazilian Al-resistant cultivar, Carazinho. Carazinho was derived from the cross of Frontana (resistant)/Egret (sensitive), and Frontana was derived from the cross Polyssu/ Alfredo Chaves 6//Fronteira/Mentana (Sasaki et al. 2002). Therefore, the major QTL for Al resistance in Atlas 66, BH 1146, and ET8 likely came from the same Brazilian source, Polyssu (Foy et al. 1965). Malate release could be a mechanism of Al resistance unique to this Brazilian source.

In addition to the QTL on 4DL, other genes have been associated with Al-resistance in Atlas 66 (Pellet et al. 1996, 1997; Basu et al. 1997). Berzonsky (1992) proposed that besides a dominant gene in the D genome, genes in genomes A and/or B might also be involved in Al resistance of Atlas 66. Tang et al. (2002) demonstrated that NILs carried only partial Al resistance from Atlas 66 and suggested that at least two QTLs may be involved in Al resistance of Atlas 66. Pellet et al. (1996, 1997) demonstrated the possibility of phosphate release from the root apex as another mechanism besides malate release. In this study, we identified an additional QTL on 3BL that accounted for 11% of the phenotypic variation for HSS, offering additional support of a multigenic model. This new QTL has not been reported previously, and it has been validated in another population with Atlas 66 as a parent, Atlas 66/Century. Therefore, the new QTL is a

stable QTL for Al resistance across different genetic backgrounds.

Both QTLs on 4DL and 3BL explained about 58% of the phenotypic variation, leaving substantial phenotypic variation still unexplained. Another unidentified QTL may be responsible for the remaining effect but went undetected in this study due to the lack of closely linked markers. Alternatively, the effect of the 3BL QTL was underestimated due to the lack of flanking markers for this QTL. Furthermore, other endogenous resistance mechanisms might condition Al resistance in wheat (Basu et al. 1999; Kidd et al. 2001; Ofei-Manu et al. 2001).

Although the QTL on 3BL provided no additional improvement in root elongation under Al stress when the 4DL QTL was present, it did offer a modest increase in root elongation when the 4DL QTL was absent (Fig. 4). Plants with the resistance allele of the 3BL QTL alone grew 1 cm longer than those with neither resistance allele, and good coincidence was found between plants with a HSS of grade 2 and plants with the resistance allele of the 3BL QTL. Altogether we can conclude that the resistance allele of the 3BL QTL partially protects root tips from toxic Al accumulation. Whether the QTL on 3BL contributes to malate release or other mechanisms is still unknown and needs investigating.

Although the *ALMT1*-specific marker can be directly used in marker-assisted breeding to select for the QTL on 4DL, analysis of this marker requires the additional costly step of restriction digestion. As an alternative, two SSR markers, Xwmc331 and Xgdm125, which flanked the 4DL QTL are suitable for high-throughput MAS. For the QTL on 3BL, although flanking markers are not available, the SSR marker Xbarc164 is a potentially useful marker for selection of Al resistance in breeding populations.

Acknowledgments This paper reports the results of research only. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. This is contribution No. 06-294-J of the Kansas Agricultural Experiment Station, Manhattan, KS, U.S.A.

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